



fish tissue TRVs

John Toll

to:

Eric Blischke, Burt Shephard

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Cc:

Chip Humphrey, Jim McKenna, Bob Wyatt, "Keith Pine (kpine@anchorenv.com)", "Jen Woronets (jworonets@anchorenv.com)", David Ashton, Matt Luxon, Lisa Saban

Hide Details

From: John Toll <JohnT@windwardenv.com> Sort List...

To: Eric Blischke/R10/USEPA/US@EPA, Burt Shephard/R10/USEPA/US@EPA,

Cc: Chip Humphrey/R10/USEPA/US@EPA, Jim McKenna <Jim.McKenna@portofportland.com>, Bob Wyatt <rjw@nwnatural.com>, "Keith Pine (kpine@anchorenv.com)" <kpine@anchorenv.com>, "Jen Woronets (jworonets@anchorenv.com)"

<jworonets@anchorenv.com>, David Ashton <david.ashton@portofportland.com>, Matt Luxon <MattL@windwardenv.com>, Lisa Saban <LisaS@windwardenv.com>

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1 Attachment



behavior reevaluation\_011409.pdf

Eric and Burt – Friday afternoon 1/9/09 the three of us and Jim McKenna met to try to resolve the final issues concerning fish tissue TRVs. That meeting was arranged to follow-up on the January 7 e-mail from Jim to Eric regarding the LWG's initial review of EPA's response to fish tissue TRVs. The January 7 e-mail identified two significant concerns that could hold-up resolution of the fish tissue TRVs. Those issues were 1) Inclusion of the Berlin et al. (1981) and Broyles & Noveck (1979) studies and 2) specific decisions regarding the use of behavioral studies. As action items coming out of our Friday meeting the LWG agreed to summarize our reevaluation of behavioral studies and our arguments for excluding Berlin et al. and Broyles & Noveck. The results of that work are provided in this e-mail and the attached table.

#### Berlin et al. (1981) and Broyles & Noveck (1979)

In our January 7 e-mail and January 9 meeting, we argued that Inclusion of the Berlin et al. (1981) and Broyles and Noveck (1979) studies is inconsistent with the LWG/EPA agreed-upon tissue TRV methodology. These studies were conducted on larvae from fish that were collected from the Great Lakes in the 1970s. Berlin et al. measured egg PCB and DDT residues (prior to initiating their experiment) and found them to be elevated (7.6 and 3.8 µg/g, respectively). Broyles and Noveck did not measure egg residues but they estimated the egg PCB residue to be in the 3-11 ppm range (which by the way turned out to be consistent with the Berlin et al. measurements that were made a couple of years later). Therefore, the controls in these studies were contaminated, and the studies are unacceptable because field collection of maternal fish resulted in contaminated test organisms.

We were unable to reach agreement on this during our January 9 meeting so the LWG went back to the papers in question in an effort to better explain why maternal transfer to the eggs that were reared to yield the test organisms used in these studies invalidates the studies, for the purpose of tissue TRV development for this BERA. Our explanations are as follows:

- Berlin et al. (1981) should be rejected because exposure and effects weren't measured at the same time. Most of the excess mortality occurred between days 17 and 96 (and virtually all of the excess mortality occurred between days 17 and 136). Tissue residues weren't measured until the end of the 176 day study. The LOER calculated at the end of the study was less than the initial tissue residue (i.e., the initial tissue concentration due to maternal transfer was higher than the final tissue concentration even though the mass of PCBs in the test organisms increase, at least in part because of growth dilution). Therefore, the LOER based on measurements taken at the end of the study is an underestimate and should be rejected.
- Broyles and Noveck (1979) should be rejected because they only measured the tissue residue of a radiolabeled fraction of the total PCB tissue residue. The experimental exposure was to <sup>14</sup>C-labeled PCB 153 and the tissue residues reported are the <sup>14</sup>C-labeled PCB 153 fraction, not the total PCB tissue residue. The authors haven't accounted for the tissue burden resulting from maternal transfer. Tissue residues were higher than reported because the test organisms were reared from eggs taken from fish captured in Lake Michigan in 1976. Our rough estimate is that the tissue burden from maternal transfer alone (at the end of the experiment, after experimental exposure and growth dilution) would add > 25% to the reported LOER. Therefore, the LOER based on measurements of radiolabeled PCB 153 is an underestimate and should be rejected.

#### Behavioral Endpoints

Following review of the Fish Tissue-Residue Toxicity Reference Value (TRV) Reconciliation Tables submitted to EPA on November 20, 2008, EPA identified exclusion of behavior studies as a primary area of disagreement with LWG regarding exclusion of studies from the species sensitivity distributions used to derive fish tissue-residue TRVs. EPA's memo dated 12/22/08 provided evidence linking prey capture ability, avoidance behavior, feeding behavior, and swimming activity to adverse effects at the population or community level. Following is the LWG's evaluation of the degree to which the evidence EPA provided for population level adverse effects described for these behaviors can be extrapolated to other studies.

##### 1. Predator-prey relationships

EPA used the example of Weiss et al. (2000) to support inclusion of studies reporting predator-prey relationships in SSDs. Weiss et al. (2000) provide literature as well as field and laboratory experimental evidence showing causal linkages between mummichog exposure to chemical contaminants (primarily mercury), reduced prey capture ability, and reduced growth and lifespan. The specific behavior reported was time required to capture prey. A possible causal mechanism that was experimentally described was increased brain mercury concentrations causing decreased neurotransmitter levels. Linkages between organism and population level traits were demonstrated for a single contaminated site where several contaminants were present. The study does a convincing job of tying reduced mummichog growth and life-span at the contaminated site investigated to decreased ability to capture prey; however, applying these results to predator-prey studies in general constitutes extrapolating outside the dataset. Nonetheless, conservatively, this study supports inclusion of studies reporting reduced prey capture rate in SSDs. More generally this study suggests that any endpoint directly related to reduced prey capture ability may influence population level endpoints. Therefore, all studies reporting prey capture rate LOERs will be included in SSDs whereas studies reporting other predator-prey

interactions will be considered on a case by case basis.

2. Avoidance behavior

No avoidance behavior studies were included in the SSD database so evidence supporting inclusion of studies reporting this category of behaviors was not reviewed.

3. Feeding behavior

EPA used the example of Weiss et al. (2000) (discussed above under predator-prey relationships) to support inclusion of studies reporting feeding behavior in SSDs. Weiss et al. (2000) report that mummichog from a contaminated site fed on detritus, whereas at an uncontaminated reference site they fed on mobile prey. No specific feeding behaviors other than prey capture rate (described above) were investigated. EPA also referenced Buckley et al. (1982) as evidence that reduced feeding by coho salmon resulted in reductions in growth. Buckley et al. (1982) reports that feeding rates were initially depressed in copper exposed fish relative to controls but recovered to control levels with time and that weight of exposed fish (except at the highest exposure level) also became similar to that of controls near the end of the 100 day aqueous copper exposure. At the highest treatment level, the observed growth effect was potentially attributable to loss of food from the tank due to reduced movement of the fish. This study demonstrates that the effect of reduced feeding rate on population level endpoints is linked to the persistence and magnitude of the reduced feeding rate effect. Based on EPA's analysis, studies reporting feeding behaviors will be individually evaluated to determine if the magnitude and duration of the effect is likely to result in reduced growth.

4. Swimming activity

EPA used the example of Smith and Weis (1997) to support inclusion of studies reporting effects on swimming activity in SSDs. Smith and Weis (1997) did not experimentally investigate any swimming behaviors. The nearest endpoints evaluated were the number of strikes by mummichog on grass shrimp made per fish per minute, the number of strikes per fish per kill, and the number of strikes per pursuit. The authors found that mummichog from a contaminated site had fewer strikes on grass shrimp than mummichog from a reference site. They suggest that a possible mechanism for this effect is related to contaminant-related changes in neurotransmitter levels causing reduced swimming activity. The evidence presented by EPA thus does not demonstrate causal relationship between swimming activity and population-level adverse effects, so it will be necessary to reevaluate swimming behavior studies on a case-by-case basis.

Our conclusions regarding the general evidence for inclusion of these types of behavioral endpoints indicates the need to reevaluate the specific papers in question on a case-by-case basis. We collectively discussed the papers and the LWG's conclusions during our January 9 meeting. The result of that discussion was your asking us for a summary of our analyses. You also asked us to be clear about which specific behavioral studies were rejected based on grounds other than use of a behavioral endpoint. That work is presented in the attached table. Twenty of the 27 LOERs have been rejected on grounds other than use of a behavioral endpoint. Of the remaining seven, we've rejected two and accepted five. The two that were rejected are Davy et al. (1972) and Webber & Haines (2003). We accept Weber et al. (1991), Kania & O'Hara (1974), Begtsson (1980) and two LOERs from Gakstatter & Weiss (1967).

- Davy et al. (1972) experimentally investigated the number of consecutive turns made by goldfish exposed to DDT versus controls. DDT exposed fish showed a significant reduction in the period of time that a pattern of turns is repeated relative to controls. No evidence has been provided linking number of consecutive turns to reduced survival, growth or reproduction. Therefore, the LWG has rejected this study for use in fish tissue TRV derivation.
- Webber and Haines (2003) report that golden shiner exposed to the highest level of mercury "had significantly greater shoal vertical dispersal following predator exposure, took longer to return to pre-exposure activity level, and had greater shoal area after return to pre-exposure activity than controls." Although the authors contend that these behaviors would increase vulnerability to predation, they do not present evidence linking these behaviors to population level effects to support their contention. Therefore the LWG has rejected this study for use in fish tissue TRV derivation.
- Webber et al. (1991) experimentally investigated feeding behavior for 14 days in fathead minnows previously exposed to dietary lead. Lead exposed minnows spent more time foraging, had more miscues, and shorter reaction distances relative to controls. Therefore this study warrants inclusion in fish tissue TRV derivation.
- Kania & O'Hara (1974) report a LOER for reduction in feeding rate and ability to capture and eat prey. The LWG has agreed to include all studies reporting prey capture rate LOERs (unless the study is rejected for established reasons unrelated to the behavioral endpoint). Therefore this study warrants inclusion in fish tissue TRV derivation.
- Begtsson (1980) Investigated ability to withstand rotary flow, and found no significant difference between PCB exposed and control fish. However the LWG has included this study in fish tissue TRV derivation based on the reported reproductive LOER.
- Gakstatter & Weiss (1967) reports goldfish and bluegill LOERs for equilibrium loss and convulsions. The primary purpose of this study was to investigate chemical bioaccumulation. Behavior effects were not experimentally investigated; however, behavioral changes were relied on to terminate exposures. Specifically, "(t)he exposures were terminated when symptoms of toxicity became severe or when the insecticide concentration in the water reached a nearly constant level." The authors note that when exposed to DDT, bluegills "exhibited severe symptoms of toxicity including convulsions and equilibrium loss" and that goldfish "developed severe symptoms of poisoning." Because the behavioral effects were severe and an integral component of the experimental design, this study warrants inclusion in fish tissue TRV derivation.

One final issue that was discussed during our January 9 meeting was the Matta et al. (2001) mercury mortality LOER. During the meeting the LWG reasserted it's position that the study should be rejected for fish tissue TRV development because the observed mortality is likely to be due to aggression between males in the experimental aquaria. EPA didn't comment on its position concerning use of the mortality LOER from Matta et al. (2001) so we consider this to still be an open question.

We ask that you please consider the information contained in this e-mail and the attached table in your final deliberations on the fish tissue TRVs. If you have any questions please don't hesitate to contact me.

John

John Toll, Ph.D.  
Partner  
Windward Environmental LLC  
200 West Mercer Street, Suite 401  
Seattle, WA 98119-3958  
(206) 812-5433

(206) 913-3292 (cell)  
[www.windwardenv.com](http://www.windwardenv.com)

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